M1D3: PCR and Paper Discussion

2/12/15

Normal OH
M 3-4 F 3-4 → 16-319

snowpocalypse!!!

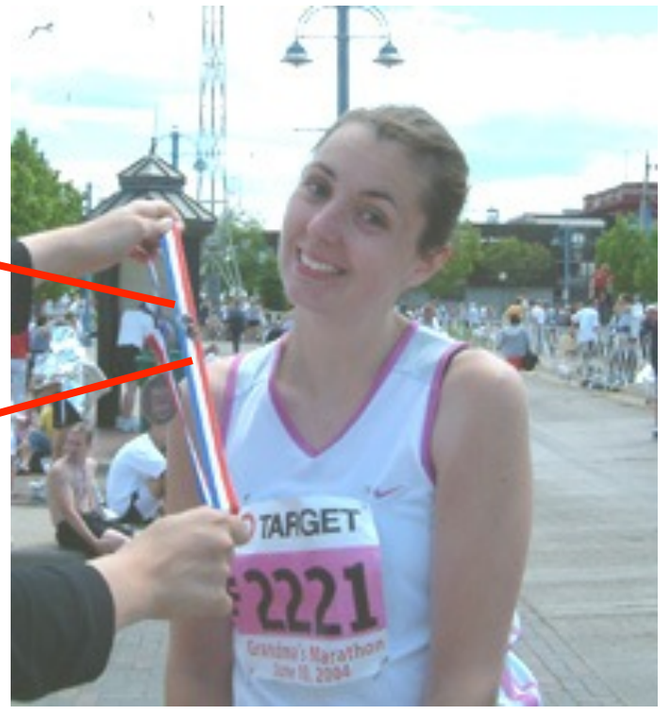
1. Upcoming office hours:
    - Tomorrow, 3-5pm + Sunday, 3-5pm in 16-319
    - No office hours on Monday (Holiday)
    - Wednesday, 3-5pm + by email ←

☆No class on Tuesday →

Announcements

- First lab treat:

Another source of DNA
for our studies



- Homework — long due to Holiday

- PCR Review, Gel Electrophoresis & Cloning basics

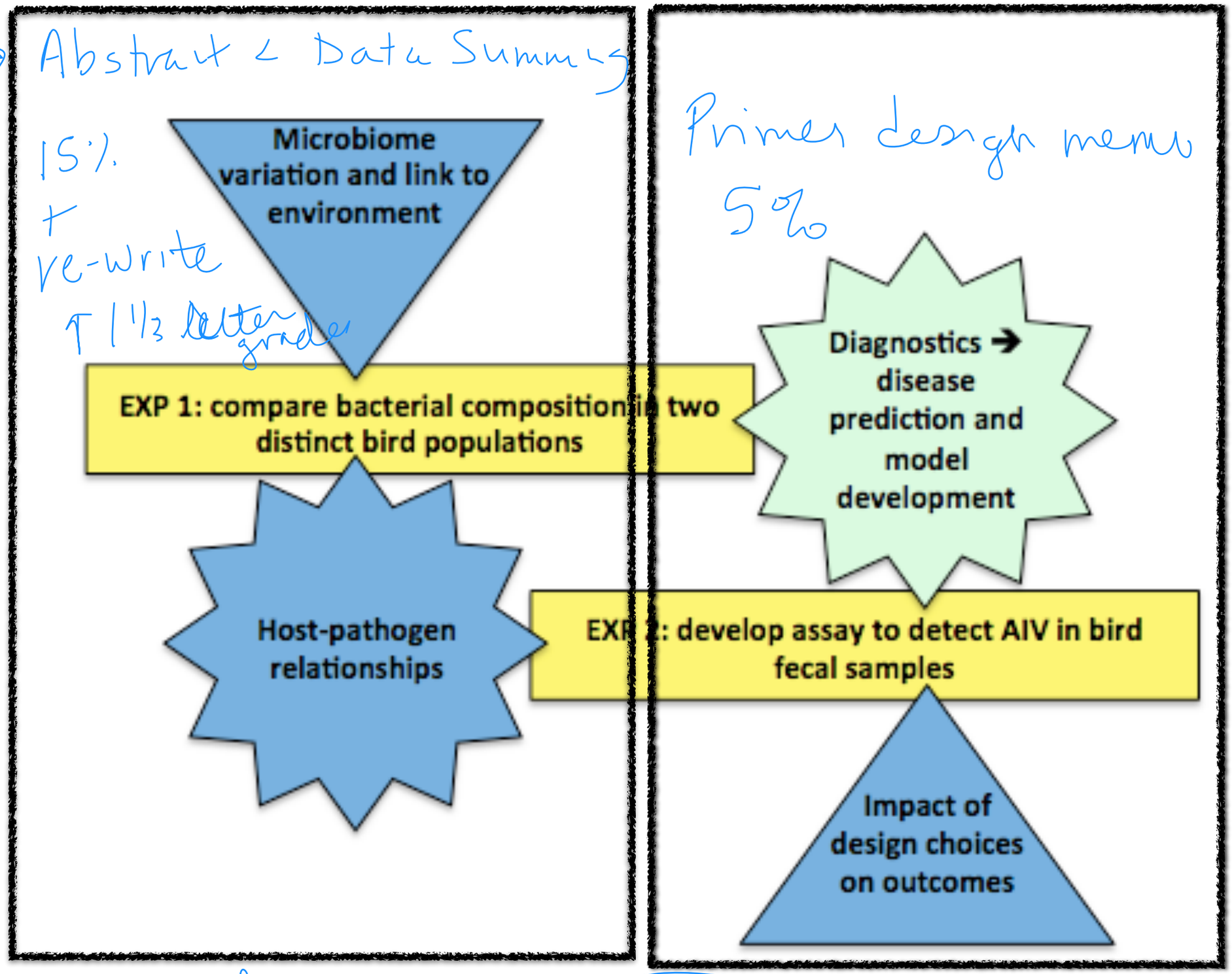
- Set up PCR

- Atissa will be here, then journal club!

Mod 1 Major Assignments

→ Abstract & Data Summary

15%
+
re-write
↑ 1½ letter grade



* Journal Club → M11/6 & M11/9

Homework Assignment

1. Larger than usual — put that long weekend to good use!
   - Experiment #1 (gull microbiota) — your own experimental schematic diagram (to be included in your *Abstract & Data Summary* assignment)
   - Experiment #1 (gull microbiota): Methods section practice — DNA purification and PCR
   - Experiment #2: (AIV detection)— a publishable table including your primer sequence and details (to be included in your *Memo* assignment)
   - M1D4 ligation calculation — Xcel Spreadsheet → input your PCR yield ⇒ output ligation parameters

A Methods section is not a protocol:

has some working Knowledge of your field

Materials and Methods [edit]

The methods section should allow an independent investigator to repeat any of your experiments. Use sub-section headings to allow readers to quickly identify experiments of interest to them (e.g., "Protein conjugation to hydrogels" or "Cell culture and fluorescent labeling"). When commercially available kits were used, it is sufficient to cite the name of the kit and say that it was used according to the manufacturer's protocol. The key to a good methods section is developing your judgement for what information is essential and what is extraneous.

Note that the methods section should be written in the past tense, since your experiments are already complete at the time you are writing your paper. This section should also be written in complete sentences and paragraphs, not in bullet point form.

1) sub section headings
"PCR" => "Amplification of bacterial 16S rRNA"

2) ** Not a protol * - generalizable → Molar quantities
→ NOT in volume
→ log-specific

3) Logical order → (MIDI + MID3)
- experiment as whole

4) cite the manufacturer (company name, location)
* 1st time you mention

Methods section exercise

- Consider the following passage: "Template DNA (5 ng) and primers were mixed with 20 uL of 2.5X Master Mix in a PCR tube. Water was added to 50 uL. A tube without template was prepared and labeled control."
  - What information is missing?
  - What information can be cut?

[2pM]

sequence

what is it?

A no template control.....

A) list the components

B) list manufacturer (Pfu M.M., Agilent, Santa Clara)

Bird Microbial Communities -- Experimental Overview

Bird Cloacal sample

extract DNA

DAY 1

Complex DNA pool (many microbial DNAs)

PCR

DAY 3

Simpler DNA pool (bacterial 16S DNA)

~400 bp

gel purify, clone and transform

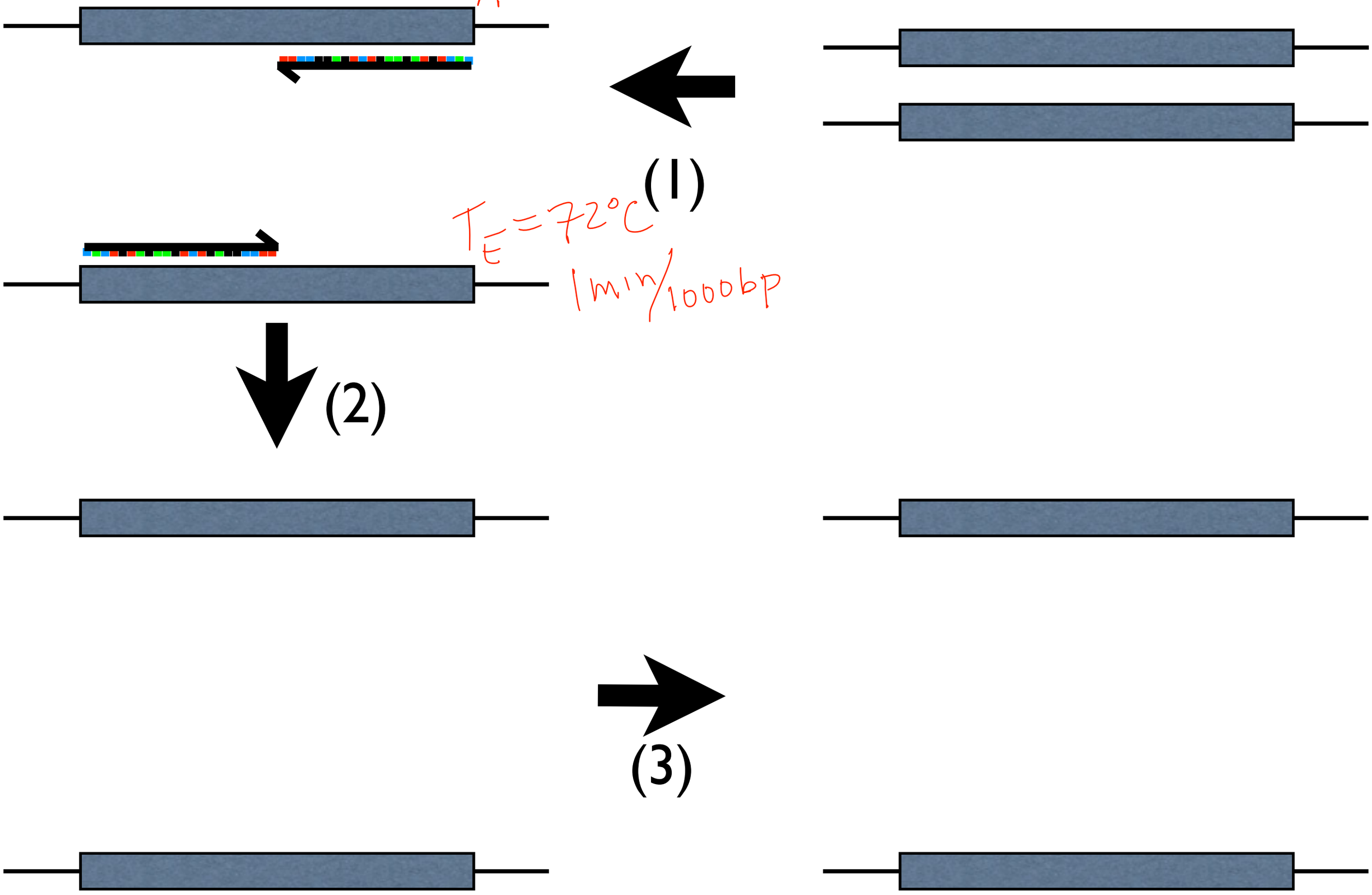
DAY 4

gel electrophoresis

Individual 16S cell colonies

First three rounds of PCR: $T_A = T_m - 5°C$

$T_A = 51°C$

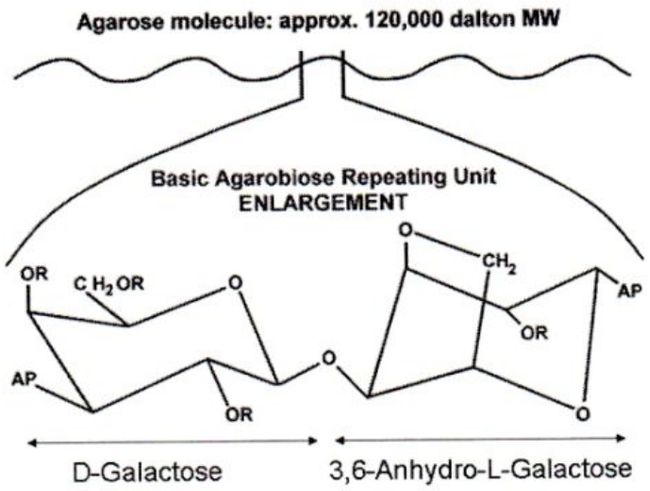


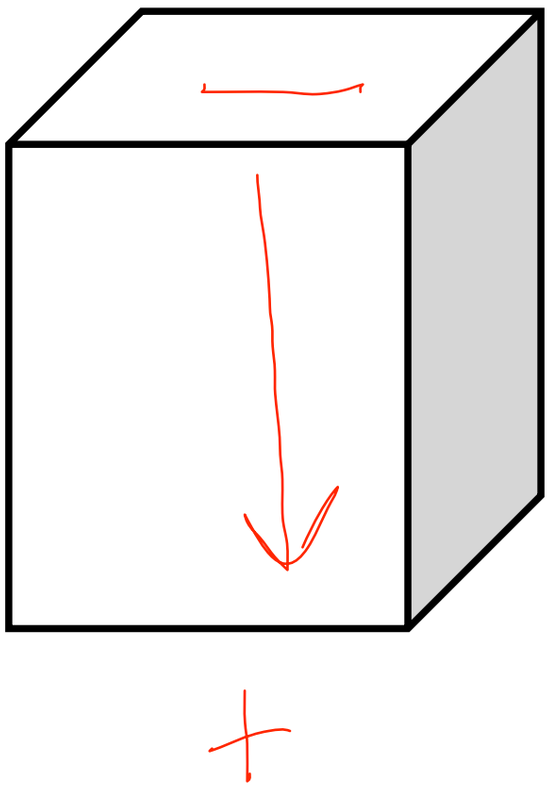
MID3: 16S rRNA gene amplification — PCR

Component	Function
Template → purified DNA MID1	Original Copy
polymerase pfu → high fidelity → hot start	Catalyzes DNA addition
dNTPs	Building Blocks
Primers	Select and initiate new sequence
Mg^{2+} Salts BSA → bovine serum albumin	Optimal chemical environment

Preview of M1D4: DNA Electrophoresis

Agarose gel





DNA

Agarose and DNA are both polymers

Driving force for separation: charge

DNA moves − to + because of negatively charged P

Separation is according to: size

Smaller DNA moves faster because

porosity

Thanks to Agi for this slide!

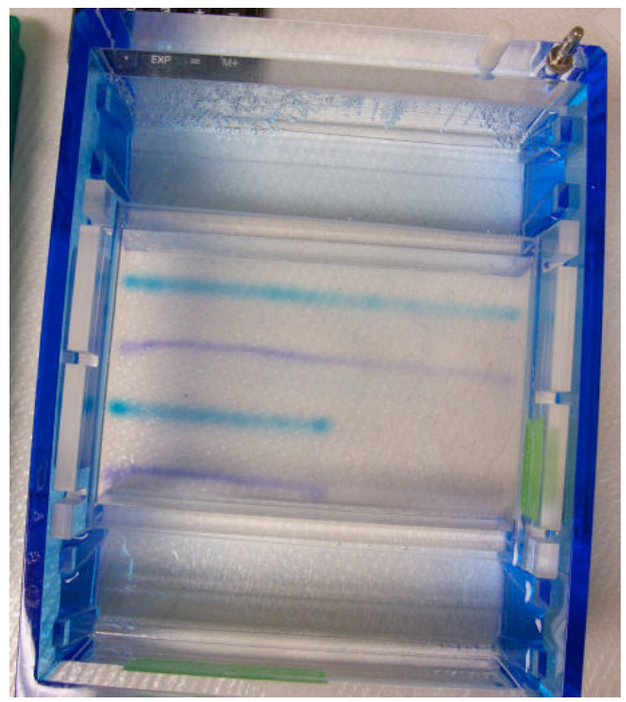
How do we visualize the DNA?

loading buffer
- glycerol
- loading dye (RNase)

UV activated dye

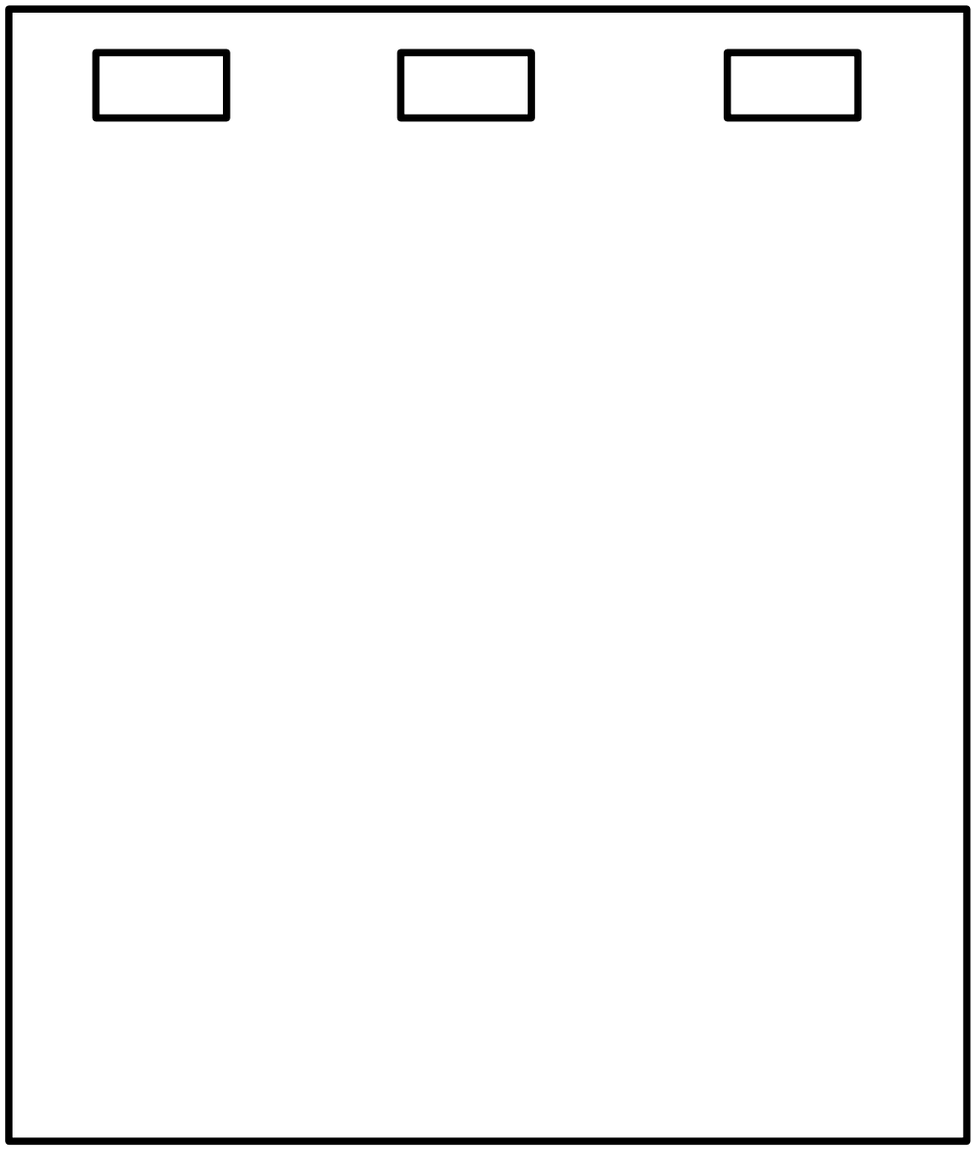
sybr safe vs. ethidium bromide

www.base-asia.com



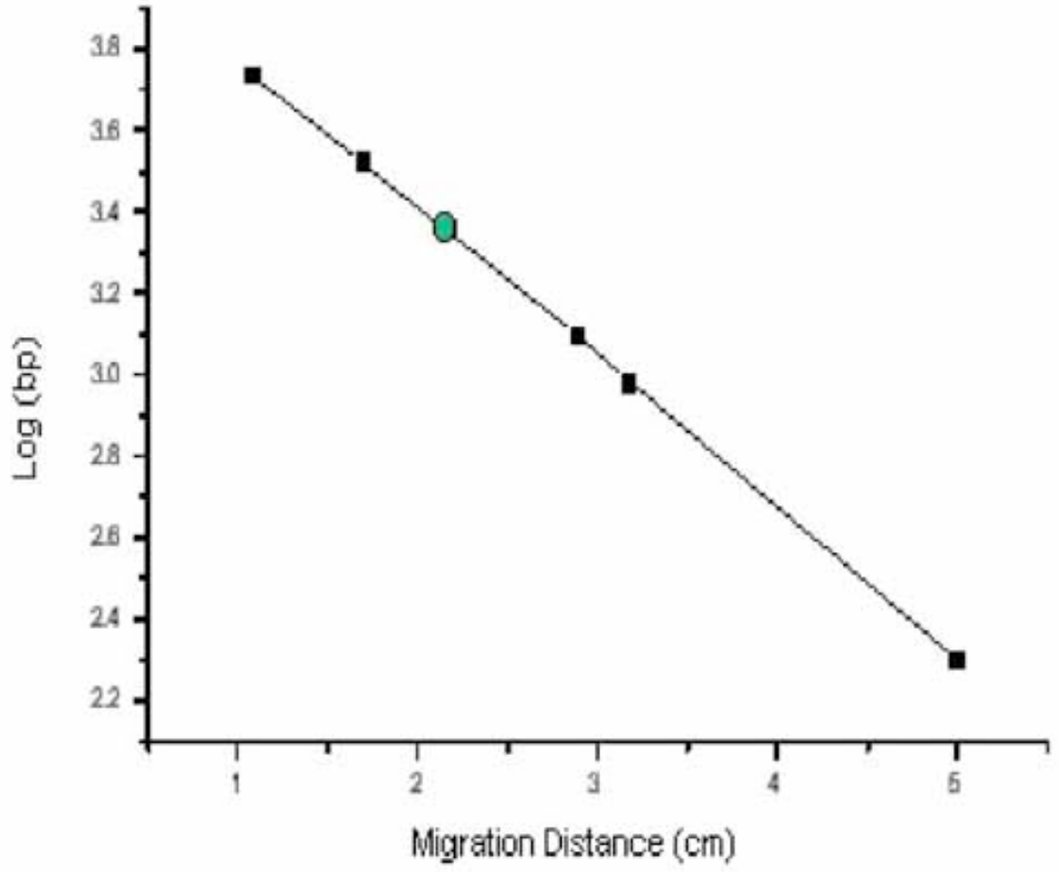
http://en.wikibooks.org/wiki/Structural_Biochemistry/DNA_Gel_Electrophoresis

Preview of M1D4: Analysis



DNA ladder:

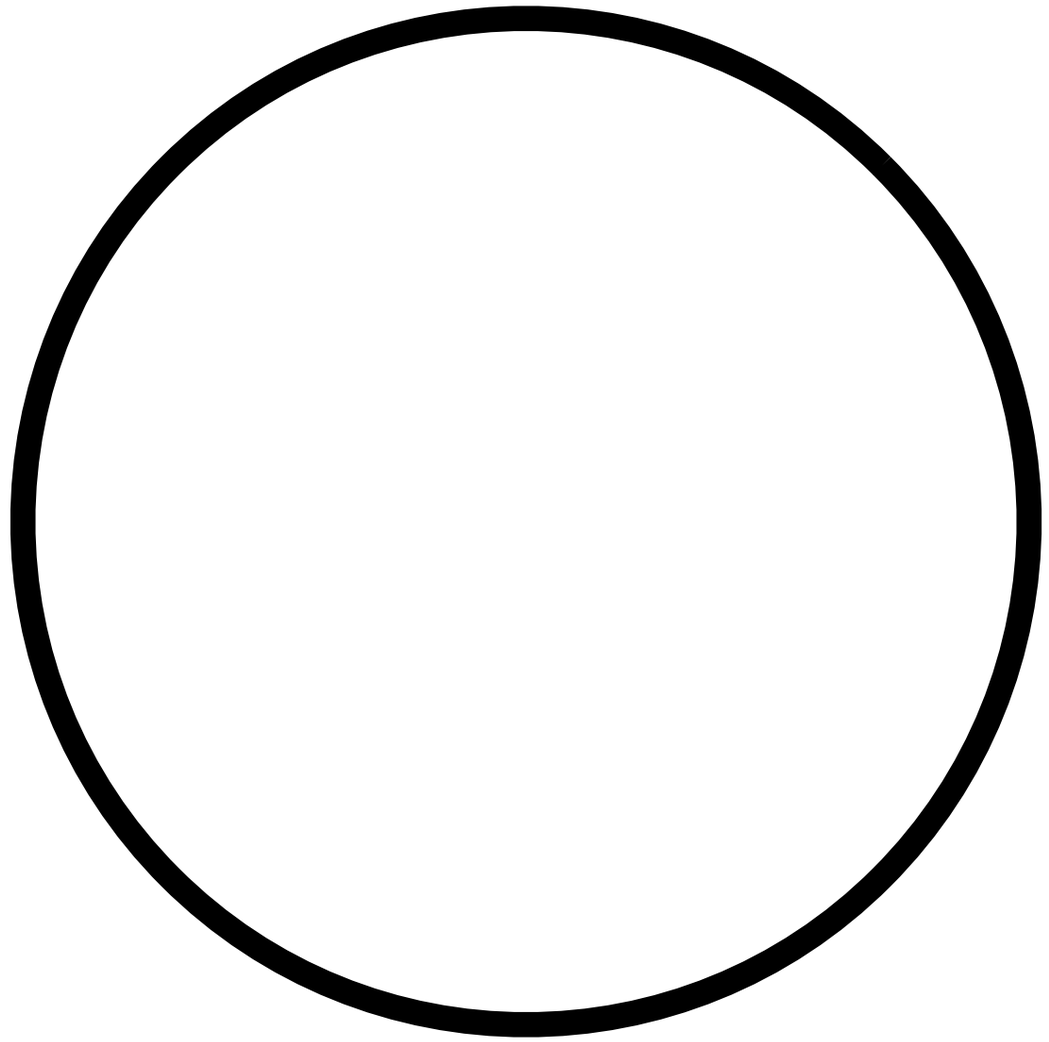
Relationship:



From MIT OCW

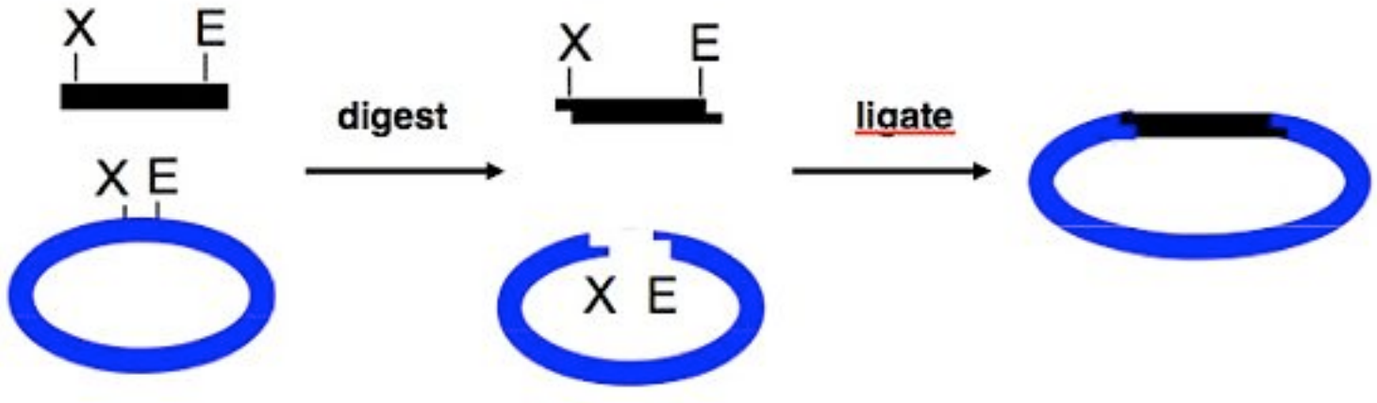
Preview of M1D4: Cloning

Vector = Plasmid = Circular DNA

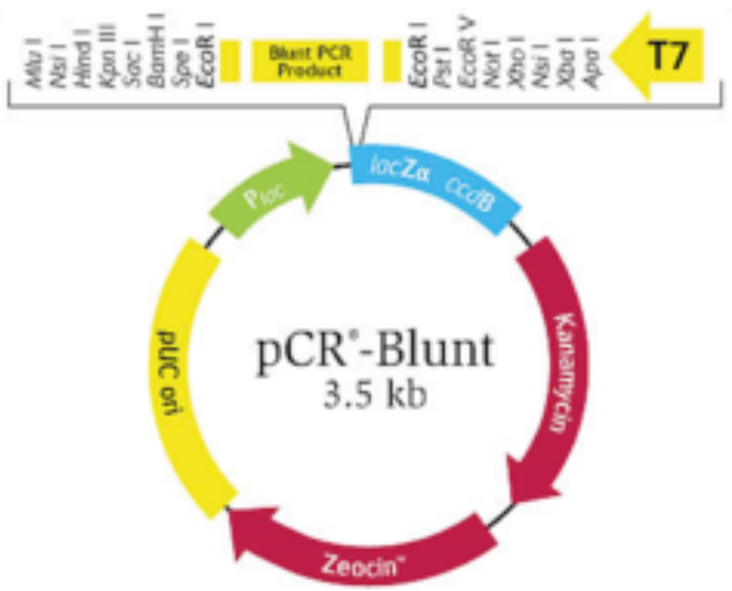


Preview of M1D4: Cloning

You may have done this before:



You can also do it this way:



Bird Microbial Communities -- Review of Overview

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extract DNA

DAY 1

Complex DNA pool (many microbial DNAs)

PCR

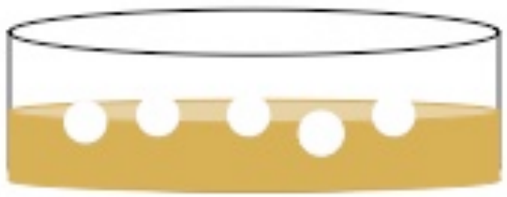
DAY 3

Simpler DNA pool (bacterial 16S DNA)

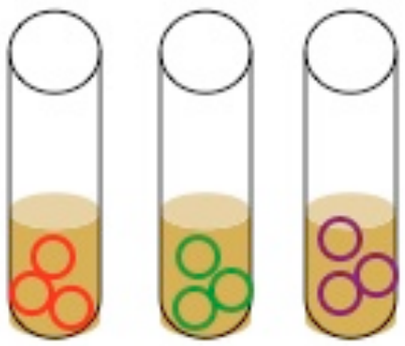
(gel purify); clone and transform

DAY 4

Individual 16S cell colonies



liquid culture



isolate DNA clones from bacteria

DAY 5

Individual 16S DNA plasmids

identify, relate, and compare sequences

DAY 7

Today in Lab: M1D3

1. Set-up PCR reactions
    - Use filtered pipette tips. Change your pipette tip after *every* step.
    - Keep the PCR tubes cold. Label with marker (not sticker!)
2. Finish up draft of slide (2 max!) for Koenig et al paper
3. Atissa here to talk Journal Club presentations (~2:45pm)
4. You 'fix' your slide based on Atissa's talk (~10-15min)
5. Class wide journal club practice (~3:45pm)
    - We will discuss the structure and content of the paper AND provide feedback on your presentation